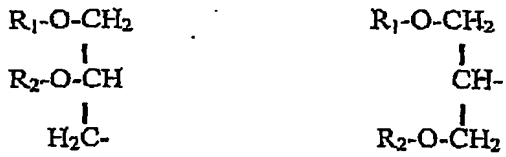


(Ia)

(Ib)

wherein R is H or a linear or branched alkyl of up to 40 carbon atoms, preferably 6-22, more preferably 10-20, and most preferably 16-20 carbon atoms; R_1 and R_2 are independently H, alkyl or acyl and wherein the alkyl or acyl groups are linear or branched having up to 40 carbon atoms, preferably 6-22, more preferably 10-20, and most preferably 16-20 carbon atoms;

B is selected from the group comprising phosphate, phosphonate, sulfonate, carbamate, and phosphothionate;

E comprises a spacer or linker group providing a linkage between groups B and D and may be selected from -cyclohexyl-; and $-CHR_3-CHR_4-$ wherein R_3 and R_4 are independently H, CH_2OH, CH_2-, $(CH(OH))_m-CH_2OH$ or

$CH((CHOH)_mCH_2OH)$-; and wherein m=1 to 6;

D comprises at least one sugar moiety selected from the group comprising D-mannose, D-galactose, D-glucose, D-glucosamine, N-acetylglucosamine, and 6-deoxy-L-mannose, wherein when D is more than one sugar moiety, the sugar moiety may comprise a single chain of the same or different sugar moieties, or may comprise two or more separate sugar moieties or chains of sugar moieties attached to E at different sites;

with the proviso that when A is a diacyl or monacyl glyceride, R_3 and R_4 cannot both be H; and with the proviso that when R_3 is H, R_4 cannot be CH_2OH.

B is selected from the group comprising phosphate, phosphonate, sulfonate, carbamate, and phosphothionate;

E comprises a spacer or linker group providing a linkage between groups B and D and may be selected from -cyclohexyl-; and $-CHR_3-CHR_4-$ wherein R_3 and R_4 are independently H, CH_2OH, CH_2- or $(CH(OH))_m-CH_2OH$ or $CH((CHOH)_mCH_2OH)$-; wherein n=1 to 40 and m=1 to 6.

D comprises at least one sugar moiety selected from the group comprising D-mannose, D-galactose, D-glucose, D-glucosamine, N-acerylglucosamine and 6-deoxy-L-mannose, wherein when D is more than one sugar moiety, the sugar moiety may comprise a single chain of the same or different sugar moieties, or may comprise two or more separate sugar moieties or chains of sugar moieties attached to E at different sites;

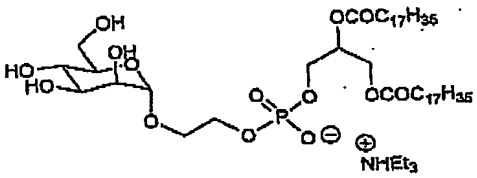
with the proviso that when A is a diacyl or monacyl glyceride, R_3 and R_4 cannot both be H; and with the proviso that when R_3 is H, R_4 cannot be CH_2OH.

Preferably, D comprises a monosaccharide or an oligosaccharide chain of 2 to 12, more preferably 2 to 6, α-1,2 and/or α-1,6 linked sugar moieties which are O-linked to carbon atoms on spacer group E. More preferably, D comprises one or more monosaccharides or oligosaccharide chains of 2 to 6 sugar moieties. One or more of the sugar moieties of D may be acylated.

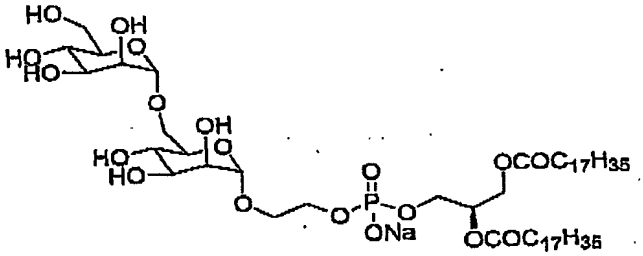
Typically, R_1 and R_2 are fatty acids independently selected from the group comprising myristate, palmitate, heptadecanoate, stearate, tuberculostearate or linolenate; B is phosphate; E is $-CHR_3CHR_4-$ where R_3 is CH_2- and R_4 is H; and D is at least one sugar moiety comprising D-mannose or oligosaccharide chain of α-1,2- and/or α-1,6- linked mannose residues.

Compounds where R, R_1 and/or R_2 comprise long chain acyl or alkyl of up to 60 carbon atoms are contemplated and may be synthesised, although synthesis may be expensive and/or

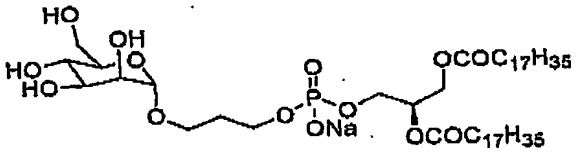
Compound 7



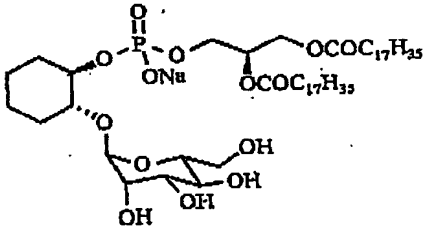
Compound 28



Compound 31



Compound 36



practitioners and other medical doctors, and typically takes account of the disorder to be treated, the condition of the individual patient, the site of delivery, the method of administration and other factors known to practitioners. Examples of the techniques and protocols mentioned above can be found in Remington's Pharmaceutical Sciences, 16th edition, Oslo, A. (ed), 1980.

In addition, it is contemplated that the compounds of the present invention may be used as an adjuvant and may be formulated into adjuvant compositions by methods well known in the art.

The present invention is also directed to a process for preparing synthetic molecules of formula (I) comprising the steps

(I) modification of a benzylated allyl glycoside compound to form an intermediate by dihydroxylation of the double bond using a catalytic amount of osmium tetraoxide and excess N-methyl morpholine-1-oxide to give a glycosyl glycerol as an intermediate for futher modification;

(II) selective benzoylation of the glycosyl glycerol intermediate to form a glycosyl glycerol unit with the 2° hydroxyl group protected as a benzoyl ester;

(III) glycosylation of the 1° hydroxyl group of the intermediate compound and selective removal of the benzoyl protecting group;

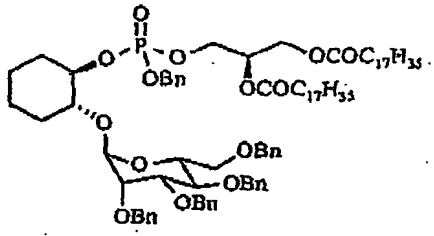
(IV) phosphorylation of the 1° or 2° hydroxyl groups of the intermediate compound;

(V) removal of the benzyl protecting groups to form a compound of formula (I).

solid. $[\alpha]_D^{28}$ 18 (*c* 1.0, CH_2Cl_2); ν_{max}/cm^{-1} 3468 br (O-H); ^1H NMR: (500MHz , $CDCl_3$) δ 7.40-7.21 (m, 16H), 7.21-7.19 (m, 4H), 5.15 (d, 1H, *J*= 2 Hz, H-1'), 4.85-4.42 (m, 8H, 4x $PhCH_2$), 4.00-3.80 (m, 3H), 3.76 (dd, 1H, *J* 11 and 5 Hz), 3.73 (dd, 1H, *J* 11 and 2 Hz), 3.69 (t, 1H, *J* 2 Hz), 3.39-3.33 (m, 1H), 3.32-3.24 (m, 1H), 2.21 (brs, 1H, OH), 2.15-2.10 (m, 1H), 1.98-1.93 (m, 2H), 1.28-1.15 (m, 4H,); ^{13}CNMR: (125 MHz , $CDCl_3$) δ 138.5, 138.5, 138.4, 138.3, 128.4, 128.4, 128.4, 128.3, 128.1, 128.0, 127.9, 127.7, 127.7, 127.7, 127.5, 99.3 (C-1'), 85.0, 79.8, 75.2, 75.2, 75.1, 73.8 73.4, 72.5, 72.4, 72.1, 69.5, 32.5, 31.4, 24.3, 24.0; HRMS-ESI(+ve) (Found: *m/z* 661.3123 (MNa^+). $C_{40}H_{46}O_7Na$ requires *m/z* 661.3141).

*-stereochemical assignment may be reversed. The C-1 and C-2 configurational assignments of **33** and **34** were made by comparison of the chemical shifts of H-1 and H-2 to those of the corresponding glucosides (Itano *et al.*, 1980).

(1*R*,2*R*)-1-*O*-(Benzyl 1,2-di-*O*-stearoyl-*sn*-glycero-3-phosphoryl)-2-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl)cyclohexane-1,2-diol **35**



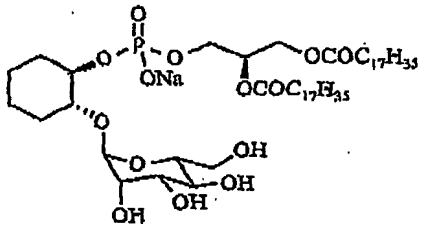
35

(structure of the bis-stearate, n=17, depicted)

A solution of the phosphoramidites **25** (185 mg, 0.215 mmol) in dichloromethane (15 mL) was cannulated onto a mixture of the alcohol **34** (115 mg, 0.180 mmol) and 1H-tetrazole (36.0 mg, 0.514 mmol) under argon. The reaction mixture was stirred at RT for 90 min. then cooled in ice when a solution of MCPBA (75%, 125 mg, 0.507 mmol), pre-dried for 20 min over $MgSO_4$, was added to the reaction mixture. After a further 20 hrs the reaction mixture was diluted with dichloromethane (30 mL) and quenched with the addition of 10% aqueous sodium thiosulfate (45 mL). The aqueous phase was further extracted with dichloromethane (35 mL) and the combined organic extract washed with sat. $NaHCO_3$ (2 x 40 mL) and dried ($MgSO_4$). After filtration the solvent was removed at reduced pressure to give the crude product that was purified by column chromatography on silica gel. Gradient elution with ethyl

acetate/petroleum ether (10:90 → 20:80) afforded the title compound **35** (90 mg, 0.064 mmol, 36%) as an inseparable mixture; ^{31}P NMR (121.2 MHz, $CDCl_3$) δ 9.2, 9.1, -0.05, -0.08; LRMS-ESI (+ve) 1434 (15%), 1406 (100), 1388 (40) 1350 (10); HRMS-ESI (+ve) (Found: *m/z* 1432.9521 (MNH_4^+). $C_{86}H_{131}NO_{14}P$ requires *m/z* 1432.9302).

1-*O*-(Sodium 1,2-di-*O*-stearoyl-*sn*-glycero-3-phosphoryl)-2-*O*-(-α-D-mannopyranosyl)-cylcohexane-1,2-diol **36**



36

(structure of the bis-stearate, n=17, depicted)

A mixture of the perbenzyl glycolipid **35** (45 mg, 0.032 mmol), $NaHCO_3$ (8.0 mg, 0.095 mmol) and Pd-black (77 mg) in ^tBuOH (3 mL) and H_2O (1.5 mL) was hydrogenated at 300 psi/50 °C for 15 hrs. After removal of the hydrogen the reaction mixture was filtered through celite and the pad washed with $CHCl_3$/MeOH/H_2O (70:40:08) (2 x 20 mL). Silica was added to the filtrate and the solvent removed at reduced pressure to give a free flowing solid. Gradient elution with $CHCl_3$ → $CHCl_3$/MeOH (80:20) → $CHCl_3$/MeOH (70:40) → $CHCl_3$/MeOH/H_2O (70:40:02) afforded the title compound **36** (11 mg, 0.011 mmol, 34%) that was lyophilized to give a white solid $[\alpha]_D^{20}$ +32 (*c* 0.55, $CHCl_3$/MeOH/H_2O, 70:40:8); ^1H NMR (300 MHz, $CDCl_3$/CD_3OD/D_2O, 70:40:6) δ 5.27-5.19 (m, 1H), 5.12 (bs, 1H), 4.42-4.37 (m, 1H), 4.20-4.14 (m, 1H), 4.50-3.48 (m, 10H), 2.38-2.30 (m, 4H), 2.12-2.03 (m, 1H), 1.99-1.90 (m, 1H), 1.70-1.55 (m, 6H), 1.40-1.20 (m 60H), 0.96-0.90 (m, 3H);^{31}P NMR (121.5 MHz, $CDCl_3$/CD_3OD/D_2O, 70:40:6) δ -0.32; HRMS-ESI(-ve) (Found: *m/z* 747.3939 $(M-Na)^-$. $C_{33}H_{64}O_{16}P$ requires *m/z* 747.3939).

Treatment protocols with PIM extract or synthetic molecules

7 to 14 days following the second i.p. injection, mice were anaesthetised. Each mouse was treated intranasally as outlined in Table 1 with the indicated concentrations of PIM extract or a synthetic molecule of the invention in 50 µl of PBS. Control mice were given PBS intranasally.

Table 1. Summary of Experiments

'PIM' Type	Dose Rates (µg 'PIM' per ml solution)	Number Of Mice
M. Bovis PIM	0, 0.02, 0.2, 2.0	17 including 5 controls*
Compound 15	0, 0.02, 0.2, 2.0	22 including 5 controls*

* The same controls (n=5) were used for each treatment group.

OVA challenge – 7 days following treatment with the test molecules, mice were anaesthetised and challenged intranasally with 50 µl of 2 mg/ml ovalbumin in PBS.

Measurements of airway eosinophilia

4 days after intranasal airway challenge with OVA the mice were sacrificed. The trachea was cannulated and bronchoalveolar lavage (BAL) was performed (3 x 1 ml PBS). Total BAL cell numbers were counted and spun onto glass slides using a cytospin. Percentages of eosinophils, macrophages, lymphocytes and neutrophils were determined microscopically using standing histological criteria.